AAC Journals.ASM.org

Metronidazole and Hydroxymetronidazole Central Nervous System Distribution: 2. Cerebrospinal Fluid Concentration Measurements in Patients with External Ventricular Drain

Denis Frasca,[a,b,c] Claire Dahyot-Fizelier,[a,b,c] Christophe Adier,[a,d] Olivier Mimoz,[a,b,c] Bertrand Debaene,[a,b,c] William Couet,[a,b,d] Sandrine Marchand[a,b,d]

Inserm U1070, Pole Biologie Santé, Poitiers, France[a]; Université de Poitiers, UFR Médecine-Pharmacie, Poitiers, France[b]; CHU Poitiers, Service d'Anesthésiologie-Réanimation Chirurgicale, Poitiers, France[c]; CHU Poitiers, Service de Toxicologie-Pharmacocinétique, Poitiers, France[d]

This study explored metronidazole and hydroxymetronidazole distribution in the cerebrospinal fluid (CSF) of brain-injured patients. Four brain-injured patients with external ventricular drain received 500 mg of metronidazole over 0.5 h every 8 h. CSF and blood samples were collected at steady state over 8 h, and the metronidazole and hydroxymetronidazole concentrations were assayed by high-pressure liquid chromatograph. A noncompartmental analysis was performed. Metronidazole is distributed extensively within CSF, with a mean CSF to unbound plasma $AUC_{0-\tau}$ ratio of 86% ± 16%. However, the concentration profiles in CSF were mostly flat compared to the plasma profiles. Hydroxymetronidazole concentrations were much lower than those of metronidazole both in plasma and in CSF, with a corresponding CSF/unbound plasma $AUC_{0-\tau}$ ratio of 79% ± 16%. We describe here for the first time in detail the pharmacokinetics of metronidazole and hydroxymetronidazole in CSF.

The blood-brain barrier (BBB) and blood-CSF barrier (BCSFB) protect the brain from neurotoxic substances and control drug distribution. Central nervous system (CNS) distribution studies are essential to predict drug's efficacy and/or side effects. Antibiotics may be used for the treatment of CNS infections but may also induce undesirable excitatory side effects. Drug distribution in the CNS has been traditionally assessed based on cerebrospinal fluid (CSF) concentrations after lumbar puncture or external ventricular drainage. However, these studies present a number of limits that have recently been reviewed (1). More recently, microdialysis was developed to measure brain extracellular fluid (ECF) concentrations of drugs as a better way to characterize CNS distribution (2). However, brain microdialysis studies in humans are relatively complex to perform, and limited data have been published (3, 4). Interestingly, recent experimental studies have shown differences between CSF and ECF concentrations for acetaminophen and quinidine in rats (5, 6). In fact, despite similarities, BBB and BSCFB present structural differences (2) that may lead to distinct brain ECF and CSF concentration-versus-time profiles. Comparisons between ECF and CSF concentrations have been reported in animals (1, 5, 6) and, to our knowledge, in one previous study on carbamazepine in humans (7). Metronidazole, a nitroimidazole antibiotic, is indicated in cerebromeningeal infections by anaerobic bacteria (8), but it is also known to induce peripheral and central side effects (9–12). Our goal was to perform a CSF distribution study in patients and to compare CSF concentrations to the ECF concentrations determined by brain microdialysis in an accompanying study (13), using metronidazole and OH-metronidazole, an active metabolite of metronidazole, as representative antibiotics with extensive CNS distribution.

MATERIALS AND METHODS

Patients. This study was performed at the University Hospital of Poitiers (France) and was approved by the local ethics committee (CPP OUEST III, protocol 2008-003311-12). Written informed consent was obtained from a legal representative for each patient. Four brain-injured male patients (three with subarachnoid hemorrhage [SAH] and one with ventricular hemorrhage [VH]) were sedated with midazolam and fentanyl and mechanically ventilated. The demographic characteristics of the patients are presented in Table 1. Patients. have undergone external ventricular drainage (EVD) using the EDS 3 CSF External Drainage System (Codman & Shurtleff, Inc., Raynham, MA) due to noninflammatory occlusive hydrocephalus.

Drug administration and sampling. Patients received metronidazole (B Braun, Boulogne Billancourt, France) and cefotaxime (Panpharma, Boulogne Billancourt, France) to treat lung infection at respective dosing regimens of 500 mg three times daily (tid) and 2 g three times daily. The metronidazole CSF pharmacokinetic study was conducted at steady state after 6 to 16 metronidazole administrations corresponding to 2 to 5 days after starting treatment. On the day of the experiment, a 30-min intravenous infusion containing 500 mg of metronidazole was started after collection of residual CSF and blood samples. Eight to twelve CSF samples and seven to ten blood samples were collected over 8 h. The blood samples were centrifuged, and plasma was collected. Two extra plasma samples were collected, one early and one later postdosing, to determine the metronidazole and OH-metronidazole unbound concentrations by ultrafiltration at 2,500 × *g* for 15 min at 4°C (Centrifree; Millipore Corp., Billerica, MA). The unbound fractions were determined for each compound and for each patient as the mean value of the unbound to total concentrations ratios estimated in these two extra plasma samples.

Metronidazole and OH-metronidazole assay. Metronidazole and OH-metronidazole concentrations were determined by high-performance liquid performance (HPLC) with UV detection (310 nm) as previously described (13). Briefly, the mobile phase was a solution of 0.01 M KH_2PO_4 mixed with acetonitrile (86/14 [vol/vol]) at a flow rate of 0.4

Received 14 August 2013 Returned for modification 2 November 2013
Accepted 20 November 2013
Published ahead of print 25 November 2013
Address correspondence to Sandrine Marchand, sandrine.marchand@univ-poitiers.fr.

doi:10.1128/AAC.01762-13

TABLE 1 Demographic characteristics of patients ($n = 4$)

Parameter	Patient			
	P1	P2	P3	P4
Age (yrs)	73	51	52	34
Sex	M	M	M	M
Ht (cm)	178	176	180	175
Wt (kg)	90	80	115	75
Creatinine clearance[a] (ml min^{-1})	165	84	141	306
Plasma proteins (g $liter^{-1}$)	61	53	66	57
No. of previous administrations	6	6	14	16
Admission type	SAH	SAH	VH	SAH

[a] Calculated by using the MDRD (modification in diet of renal disease) equation.

ml/min. Brain dialysates and ultrafiltrate (UF) samples were injected directly onto an Xterra C_{18} column after dilution with an internal standard solution of dimetridazole. The dialysate and UF metronidazole and OH-metronidazole intra- and interday variabilities were respectively characterized at four (0.25, 0.5, 2, and 20 μg ml^{-1}) and three (0.5, 2, and 15 μg ml^{-1}) concentrations and were always <15%. Plasma samples were precipitated by using acetonitrile containing an internal standard. The plasma intra- and interday variabilities were respectively characterized at four (1, 2, 10, and 40 μg ml^{-1}) and three (1, 5, and 30 μg ml^{-1}) concentrations and were always <15%.

Noncompartmental analysis. Unbound plasma concentrations were used for pharmacokinetic analysis, whereas binding was considered to be negligible in CSF (14). Individual noncompartmental pharmacokinetic analysis was performed (Phoenix WinNonlin version 6.2; Pharsight, St. Louis, MO) as previously described (13). The areas under the plasma and brain ECF unbound concentration-time curves from dosing time to the dosing interval ($AUC_{0-\tau}$) were calculated using the linear trapezoidal rule. The elimination rate constant k_e and the corresponding half-lives ($t_{1/2}$) were determined by a least-squares fit of data points (log concentration time) in the terminal phase of the decline. The steady-state unbound body clearance of cefotaxime ($CL_{ss,u}$) and volume of distribution ($V_{ss,u}$) were calculated according to standard procedures. The results are expressed as means ± the standard deviations.

RESULTS

The estimated mean unbound fractions (f_u) of metronidazole and OH-metronidazole in plasma were 89.3% ± 5.6% and 88.0% ± 6.9%, respectively, and were independent of concentrations. The individual unbound plasma and CSF metronidazole and OH-metronidazole profiles are presented on Fig. 1. Metronidazole unbound concentrations at steady-state fluctuated between mean maximal and minimal concentrations of 21.7 ± 7.1 and 7.7 ± 4.4 μg ml^{-1}, with an average concentration ($C_{average} = AUC_{0-\tau}/\tau$) of 12.7 ± 4.5 μg ml^{-1} in plasma, whereas in CSF concentrations the profiles did not exhibit a clear peak and were mostly flat, with an average steady-state unbound concentration of 11.0 ± 5.0 μg ml^{-1} (Fig. 1). The mean unbound metronidazole CSF/unbound plasma $AUC_{0-\tau}$ ratio was 86% ± 16% (Table 2). For OH-metronidazole, the unbound plasma concentrations represent only 20% of the corresponding metronidazole values, which is unlikely to contribute significantly to the antimicrobial effect. Both unbound plasma and CSF concentration-versus-time profiles were virtually flat (Fig. 1), and the mean unbound OH-metronidazole CSF to unbound plasma $AUC_{0-\tau}$ ratio was 79% ± 16% (Table 2). All of the pharmacokinetic parameters are presented in Table 2.

DISCUSSION

The metronidazole systemic pharmacokinetic parameters values (Table 2) are consistent with those estimated during the microdialysis study (13). Although CNS distribution of metronidazole is generally considered to be extensive (15, 16), this is the first

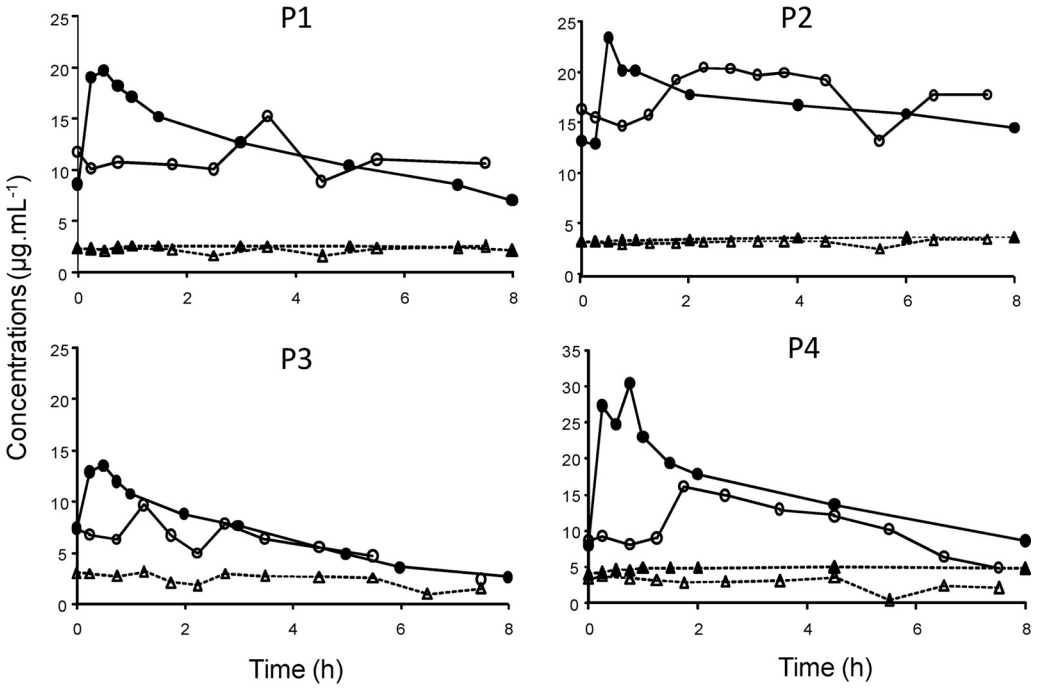

FIG 1 Individual steady-state unbound plasma concentrations of metronidazole (●, full line), OH-metronidazole (▲, dashed line), CSF metronidazole (○, full line), and hydroxymetronidazole (△, dashed line) concentrations after a 500-mg metronidazole infusion over 30 min every 8 h in critical care patients. In patient 3, the unbound plasma concentrations of OH-metronidazole were not determined due to analytical interference.

TABLE 2 Pharmacokinetic parameters determined at steady state by noncompartmental analysis in critical care patients receiving 30-min intravenous infusions of 500 mg of metronidazole every 8 h

Patient[a]	Metronidazole pharmacokinetic parameter			CSF/unbound plasma $AUC_{0-\tau}$ ratio	
	$V_{ss,u}$ (liters)	$CL_{ss,u}$ (liters h^{-1})	$t_{1/2,\ plasma}$ (h)	Metronidazole	OH-metronidazole
Individual patients					
P1	43	5.2	6.1	0.91	0.87
P2	89	3.7	16.4	1.05	0.89
P3	42	9.5	3.1	0.80	NA[b]
P4	31	4.1	5.7	0.67	0.61
Mean data					
Mean	51	5.6	7.8	0.86	0.79
SD	26	2.6	5.9	0.16	0.16

[a] Both individual and mean patient data are presented.
[b] NA, not available.

study to explore the CSF distribution of metronidazole and OH-metronidazole in brain-injured patients with EVD and uninflamed meninges. Previously published studies on metronidazole CSF penetration in human (17–22) have mostly been performed using nonspecific microbiological assay procedures, thus not distinguishing between metronidazole and OH-metronidazole (17–20). Nevertheless, a first case report in adult with meningitis, using HPLC as the analytical method, determined the metronidazole CSF concentration after the administration of 500 mg/6 h, but concomitant plasma concentrations were not reported (22). A second case report in a premature infant with bacterial meningitis reported results consistent with ours, in particular with a CSF/plasma concentration ratio close to 1 (21).

In the present study, metronidazole demonstrates an extensive penetration through BCSFB, as suggested through the BBB using brain microdialysis, when a mean metronidazole unbound brain to plasma $AUC_{0-\tau}$ ratio of 102% ± 19% was found (13). These results are consistent with the fact that metronidazole is not known to be a substrate of efflux transport systems at the CNS level. For OH-metronidazole, the mean CSF/plasma $AUC_{0-\tau}$ ratio was 79% ± 16% (Table 2). However, the metronidazole concentration profiles in ECF and CSF were different. Indeed, concentration-time curves in brain ECF were delayed compared to unbound plasma concentration-time curves, but a peak was still visible within ECF, with a mean maximal concentration equal to 14.5 ± 1.2 μg ml^{-1} (13), whereas the CSF concentration-versus-time profiles were essentially flat, with a mean steady-state concentration close to 11 μg ml^{-1}. Consequently, it was possible to estimate a metronidazole half-life in brain ECF by microdialysis (4.5 ± 0.2 h) that was not significantly different from that in plasma (6.3 ± 2.2 h) (13), whereas the metronidazole half-life in CSF could not be determined. Moreover, the absence of a peak is likely to have consequences on the antimicrobial efficacy of concentration-dependent antibiotics such as metronidazole, where the peak level and AUC parameters determine antimicrobial *in vivo* efficacy (23), as well as on excitatory side effects.

It should also be mentioned that differences observed between CSF and ECF metronidazole profiles must be interpreted with caution. First, although interpatient variability was not particularly large, the present study was conducted with a small number of patients ($n = 4$). However, and more importantly, differences observed between ECF and CSF profiles during these two studies could be due to differences between BBB and BCSFB, since respective patients also differed in terms of their physiopathology. Only patients recruited in this CSF distribution study required EVD for intracranial hypertension, and these hemodynamic alterations may influence BCSFB permeability, CSF turnover, and eventually the metronidazole CSF distribution. Furthermore, EVD itself constitutes an extra route of drug elimination. Physiologically based pharmacokinetic approaches will be used to clarify these issues.

This study is the first to describe the pharmacokinetics of metronidazole and OH-metronidazole in CSF. Metronidazole and OH-metronidazole are distributed extensively within CSF, but the concentration-versus-time profiles are relatively flat compared to the brain ECF concentrations estimated by microdialysis (13). Metronidazole penetrates extensively within the brain, a finding consistent with passive diffusion. However, CSF and ECF concentration-versus-time profiles are not superimposable, and therefore CSF distribution studies should not be used to predict the concentrations of these type of drugs in brain tissue.

ACKNOWLEDGMENT

This study was support by a PHRC grant from the French Ministère de l'Education et de la Recherche.

REFERENCES

1. **de Lange EC.** 2013. Utility of CSF in translational neuroscience. J. Pharmacokinet. Pharmacodyn. **40:**315–326. http://dx.doi.org/10.1007/s10928-013-9301-9.
2. **Westerhout J, Danhof M, De Lange EC.** 2011. Preclinical prediction of human brain target site concentrations: considerations in extrapolating to the clinical setting. J. Pharm. Sci. **100:**3577–3593. http://dx.doi.org/10.1002/jps.22604.
3. **Dahyot-Fizelier C, Timofeev I, Marchand S, Hutchinson P, Debaene B, Menon D, Mimoz O, Gupta A, Couet W.** 2010. Brain microdialysis study of meropenem in two patients with acute brain injury. Antimicrob. Agents Chemother. **54:**3502–3504. http://dx.doi.org/10.1128/AAC.01725-09.
4. **Dahyot-Fizelier C, Frasca D, Gregoire N, Adier C, Mimoz O, Debaene B, Couet W, Marchand S.** 2013. Microdialysis study of cefotaxime cerebral distribution in patients with acute brain injury. Antimicrob. Agents Chemother. **57:**2738–2742. http://dx.doi.org/10.1128/AAC.02570-12.
5. **Westerhout J, Ploeger B, Smeets J, Danhof M, de Lange EC.** 2012. Physiologically based pharmacokinetic modeling to investigate regional brain distribution kinetics in rats. AAPS J. **14:**543–553. http://dx.doi.org/10.1208/s12248-012-9366-1.
6. **Westerhout J, Smeets J, Danhof M, de Lange EC.** 2013. The impact of P-gp functionality on non-steady-state relationships between CSF and brain extracellular fluid. J. Pharmacokinet. Pharmacodyn. **40:**327–342. http://dx.doi.org/10.1007/s10928-013-9314-4.

7. **Rambeck B, Jurgens UH, May TW, Pannek HW, Behne F, Ebner A, Gorji A, Straub H, Speckmann EJ, Pohlmann-Eden B, Loscher W.** 2006. Comparison of brain extracellular fluid, brain tissue, cerebrospinal fluid, and serum concentrations of antiepileptic drugs measured intraoperatively in patients with intractable epilepsy. Epilepsia **47:**681–694. http://dx.doi.org/10.1111/j.1528-1167.2006.00504.x.
8. **Shin SH, Kim KS.** 2012. Treatment of bacterial meningitis: an update. Expert Opin. Pharmacother. **13:**2189–2206. http://dx.doi.org/10.1517/14656566.2012.724399.
9. **Bernstein LH, Frank MS, Brandt LJ, Boley SJ.** 1980. Healing of perineal Crohn's disease with metronidazole. Gastroenterology **79:**599.
10. **Kim E, Na DG, Kim EY, Kim JH, Son KR, Chang KH.** 2007. MR imaging of metronidazole-induced encephalopathy: lesion distribution and diffusion-weighted imaging findings. Am. J. Neuroradiol. **28:**1652–1658. http://dx.doi.org/10.3174/ajnr.A0655.
11. **Patel K, Green-Hopkins I, Lu S, Tunkel AR.** 2008. Cerebellar ataxia following prolonged use of metronidazole: case report and literature review. Int. J. Infect. Dis. **12:**e111–e114. http://dx.doi.org/10.1016/j.ijid.2008.03.006.
12. **Seok JI, Yi H, Song YM, Lee WY.** 2003. Metronidazole-induced encephalopathy and inferior olivary hypertrophy: lesion analysis with diffusion-weighted imaging and apparent diffusion coefficient maps. Arch. Neurol. **60:**1796–1800. http://dx.doi.org/10.1001/archneur.60.12.1796.
13. **Frasca D, Dahyot-Fizelier C, Adier C, Mimoz O, Debaene B, Couet W, Marchand S.** 2014. Metronidazole and hydroxymetronidazole central nervous system distribution: 1. microdialysis assessment of brain extracellular fluid concentrations in patients with acute brain injury. Antimicrob. Agents Chemother. **58:**1019–1023. http://dx.doi.org/10.1128/AAC.01760-13.
14. **Regeniter A, Kuhle J, Mehling M, Moller H, Wurster U, Freidank H, Siede WH.** 2009. A modern approach to CSF analysis: pathophysiology, clinical application, proof of concept and laboratory reporting. Clin. Neurol. Neurosurg. **111:**313–318. http://dx.doi.org/10.1016/j.clineuro.2008.12.004.
15. **Lau AH, Lam NP, Piscitelli SC, Wilkes L, Danziger LH.** 1992. Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infectives. Clin. Pharmacokinet. **23:**328–364. http://dx.doi.org/10.2165/00003088-199223050-00002.
16. **Lamp KC, Freeman CD, Klutman NE, Lacy MK.** 1999. Pharmacokinetics and pharmacodynamics of the nitroimidazole antimicrobials. Clin. Pharmacokinet. **36:**353–373. http://dx.doi.org/10.2165/00003088-199936050-00004.
17. **O'Grady LR, Ralph ED.** 1976. Anaerobic meningitis and bacteremia caused by *Fusobacterium* species. Am. J. Dis. Children **130:**871–873.
18. **Feldman WE.** 1976. *Bacteroides fragilis* ventriculitis and meningitis: Report of two cases. Am. J. Dis. Child. **130:**880–883. http://dx.doi.org/10.1001/archpedi.1976.02120090090017.
19. **Jokipii AM, Myllyla VV, Hokkanen E, Jokipii L.** 1977. Penetration of the blood brain barrier by metronidazole and tinidazole. J. Antimicrob. Chemother. **3:**239–245. http://dx.doi.org/10.1093/jac/3.3.239.
20. **Davies AH.** 1967. Metronidazole in human infections with syphilis. Br. J. Vener. Dis. **43:**197–200.
21. **Warner JF, Perkins RL, Cordero L.** 1979. Metronidazole therapy of anaerobic bacteremia, meningitis, and brain abscess. Arch. Intern. Med. **139:**167–169. http://dx.doi.org/10.1001/archinte.139.2.167.
22. **Soriano F, Aguado JM, Tornero J, Fernandez-Guerrero ML, Gomez-Garces JL.** 1986. *Bacteroides fragilis* meningitis successfully treated with metronidazole after a previous failure with thiamphenicol. J. Clin. Microbiol. **24:**472–473.
23. **Craig WA.** 2007. Pharmacodynamics of antimicrobials: general concepts and applications, p 1–19. *In* Nightingale CH, Ambrose PG, Drusano GL, Murakawa T (ed), Antimicrobial pharmacodynamics in theory and clinical practice, 2nd ed. Informa Healthcare, New York, NY.